



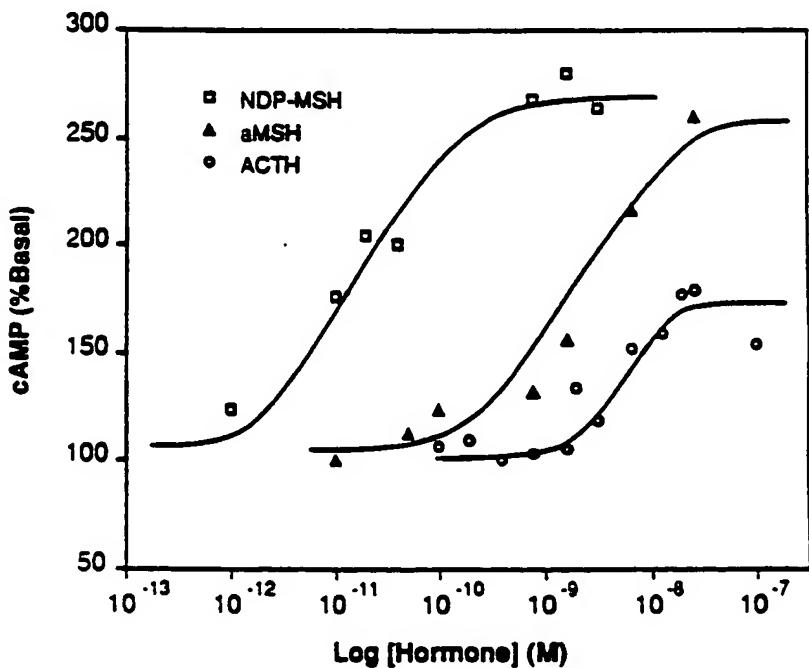
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(54) Title: MAMMALIAN MELANOCYTE STIMULATING HORMONE RECEPTORS AND USES

(57) Abstract

The present invention relates to a mammalian melanocyte stimulating hormone receptor. The invention is directed toward the isolation, characterization and pharmacological use of mammalian melanocyte stimulating hormone receptor, the gene corresponding to this receptor, a recombinant eukaryotic expression construct capable of expressing a mammalian melanocyte stimulating hormone receptor in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize mammalian melanocyte stimulating hormone receptor. The invention also provides methods for screening MSH^R agonists and antagonists *in vitro* using preparations of receptor from such cultures of eukaryotic cells transformed with a recombinant eukaryotic expression construct comprising the MSH^R receptor gene. The invention specifically provides human and mouse MSH^R genes.



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**MAMMALIAN MELANOCYTE STIMULATING HORMONE
RECEPTORS AND USES**

BACKGROUND OF THE INVENTION

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This invention was made with government support under 1R01DK41921-03, 1R01DK43859-01, and 1P01DK44239-10A1 by the National Institutes of Health. The government has certain rights in the invention.

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1. Field of the Invention

This invention relates to melanocyte stimulating hormone receptors from mammalian species and the genes corresponding to such receptors. Specifically, the invention relates to the isolation, cloning and sequencing of a human melanocyte stimulating hormone receptor gene. The invention also relates to the isolation, cloning and sequencing of a mouse melanocyte stimulating hormone receptor gene. The invention relates to the construction of eukaryotic recombinant expression constructs capable of expressing these melanocyte stimulating hormone receptors in cultures of transformed eukaryotic cells, and the production of the melanocyte stimulating hormone receptor in such cultures. The invention relates to the use of such cultures of transformed eukaryotic cells to produce homogeneous compositions of such melanocyte stimulating hormone receptors. The invention also provides cultures of such cells producing melanocyte stimulating hormone receptor for the characterization of novel and useful drugs. Antibodies against and epitopes of these melanocyte stimulating hormone receptor proteins are also provided by the invention.

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2. Background of the Invention

The proopiomelanocortin (POMC) gene product is processed to produce a large number of biologically active peptides. Two of these peptides, α -melanocyte stimulating hormone (α MSH), and adrenocorticotrophic hormone (ACTH) have well understood roles in control of melanocyte and adrenocortical function, respectively. Both of these hormones, however, are found in a variety of forms with unknown functions. The melanocortin peptides also have a diverse

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array of biological activities in other tissues, including the brain, and immune system, and bind to specific receptors there with a distinct pharmacology [see, Hanneman *et al.*, in *Peptide Hormone as Prohormones*, G. Martinez, ed. (Ellis Horwood Ltd.: Chichester, UK) pp. 53-82; DeWied & Jolles, 1982, *Physiol. Rev.* 62: 976-1059 for reviews].

5 A complete understanding of these peptides and their diverse biological activities requires the isolation and characterization of their corresponding receptors. Some biochemical studies have been reported on the prior art.

10 Shimuze, 1985, *Yale J. Biol. Med.* 58: 561-570 discusses the physiology of melanocyte stimulating hormone.

Tatro & Reichlin, 1987, *Endocrinology* 121: 1900-1907 disclose that MSH receptors are widely distributed in rodent tissues.

15 Solca *et al.*, 1989, *J. Biol. Chem.* 264: 14277-14280 disclose the molecular weight characterization of mouse and human MSH receptors linked to radioactively and photoaffinity labeled MSH analogues.

Siegrist *et al.*, 1991, *J. Receptor Res.* 11: 323-331 disclose the quantification of receptors on mouse melanoma tissue by receptor autoradiography.

20 The present invention comprises a human melanocyte stimulating hormone receptor gene, the nucleotide sequence of this gene and the deduced amino acid sequence of its cognate protein, a homogeneous composition of the melanocyte stimulating hormone receptor, nucleic acid hybridization probes and a method for determining the tissue distribution of expression of the gene, a recombinant expression construct capable of expressing the gene in cultures of transformed eukaryotic cells, and such cultures of transformed eukaryotic cells useful in the characterization of novel and useful drugs. The present invention also comprises the homologue of the human melanocyte stimulating hormone receptor gene from the mouse.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the nucleotide sequence of the mouse (SEQ ID NO:3) and human (SEQ ID NO:5) melanocyte stimulating hormone receptor.

5 Figure 2 presents an amino acid sequence comparison between the mouse
and human melanocyte stimulating hormone receptor proteins.

Figure 3 illustrates binding of melanocyte stimulating hormone receptor agonists to mouse melanocyte stimulating hormone receptor expressed in human 293 cells.

Figure 4 illustrates the tissue distribution of human (Panel A) and mouse (Panel B) melanocyte stimulating hormone receptor gene expression by Northern blot hybridization.

SUMMARY OF THE INVENTION

The present invention relates to the cloning, expression and functional characterization of mammalian melanocyte stimulating hormone receptor (MSH^R) genes. The invention comprises the nucleotide sequence of these genes encoding the mammalian MSH^R's and the deduced amino acid sequences of the cognate proteins, as well as tissue distribution patterns of expression of these genes.

In particular, the present invention is directed toward the isolation, characterization and pharmacological use of the human MSH^R, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the human MSH^R, a recombinant eukaryotic expression construct capable of expressing the human MSH^R in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the human MSH^R, a homogeneous composition of the human MSH^R, and antibodies against and epitopes of the human MSH^R.

The present invention is also directed toward the isolation, characterization and pharmacological use of the mouse MSH^R, the gene corresponding to this receptor, a nucleic acid hybridization probe comprising DNA sequences of the mouse MSH^R, a recombinant eukaryotic expression construct capable of expressing the mouse MSH^R in cultures of transformed eukaryotic cells and such cultures of transformed eukaryotic cells that synthesize the mouse MSH^R, a homogeneous composition of the mouse MSH^R, and antibodies against and epitopes of the mouse MSH^R.

It is an object of the invention to provide a nucleic acid comprising a nucleotide sequence encoding a mammalian MSH^R. In a preferred embodiment of the invention, the nucleotide sequence encodes the human MSH^R. In another preferred embodiment, the nucleotide sequence encodes the mouse MSH^R.

The present invention includes a nucleic acid comprising a nucleotide sequence encoding a human MSH^R receptor derived from a DNA molecule isolated from a human genomic library (SEQ ID NO:5). In this embodiment of the invention, the nucleotide sequence includes 1635 nucleotides of the human MSH^R gene comprising 953 nucleotides of coding sequence, 462 nucleotides of

5' untranslated sequence and 220 nucleotides of 3' untranslated sequence.

The present invention also includes a nucleic acid comprising a nucleotide sequence encoding a mouse MSH^R derived from a cDNA molecule isolated from a CDNA library constructed with RNA from mouse Cloudman melanoma cells (SEQ ID NO:3). In this embodiment of the invention, the nucleotide sequence includes 1260 nucleotides of the mouse MSH^R gene comprising 947 nucleotides of coding sequence, 15 nucleotides of 5' untranslated sequence and 298 nucleotides of 3' untranslated sequence.

The invention includes nucleic acids comprising the nucleotide sequences of mammalian MSH^Rs, most preferably mouse and human MSH^Rs (SEQ ID NOs:3&5), and includes allelic variations of these nucleotide sequences and the corresponding MSH^R molecule, either naturally occurring or the product of *in vitro* chemical or genetic modification, each such variant having essentially the same nucleotide sequence as the nucleotide sequence of the corresponding MSH^R disclosed herein, wherein the resulting MSH^R molecule has substantially the same biological properties as the MSH^R molecule corresponding to the nucleotide sequence described herein. The term "substantially homologous to" as used in this invention encompasses such allelic variability as described in this paragraph.

The invention also includes a protein comprised of a predicted amino acid sequence for the mouse (SEQ ID NO:4) and human (SEQ ID NO:6) MSH^R deduced from the nucleotide sequence comprising the complete coding sequence of the mouse (SEQ ID NO:3) and human (SEQ ID NO:5) MSH^R gene as described herein.

In another aspect, the invention comprises a homogeneous composition of a 35.3 kilodalton mouse MSH^R or derivative thereof, wherein the amino acid sequence of the MSH^R or derivative thereof comprises the mouse MSH-R sequence shown in Figure 2 (SEQ ID NO:4).

In another aspect, the invention comprises a homogeneous composition of a 34.7 kilodalton human MSH^R or derivative thereof, wherein the amino acid sequence of the MSH^R or derivative thereof comprises the human MSH-R sequence shown in Figure 2 (SEQ ID NO:6)..

This invention provides both nucleotide and amino acid probes derived from these sequences. The invention includes probes isolated from either cDNA or genomic DNA clones, as well as probes made synthetically with the sequence information derived therefrom. The invention specifically includes but is not limited to oligonucleotide, nick-translated, random primed, or *in vitro* amplified probes made using cDNA or genomic clone embodying the invention, and oligonucleotide and other synthetic probes synthesized chemically using the nucleotide sequence information of cDNA or genomic clone embodiments of the invention.

It is a further object of this invention to provide sequences of mammalian MSH^R, preferably the mouse or human MSH^R, for use as nucleic acid hybridization probes to determine the pattern, amount and extent of expression of this receptor in various tissues of mammals, including humans. It is also an object of the present invention to provide nucleic acid hybridization probes derived from the sequences of the mouse or human MSH^R to be used for the detection and diagnosis of genetic diseases. It is an object of this invention to provide nucleic acid hybridization probes derived from the DNA sequences of the mouse or human MSH^R to be used for the detection of novel related receptor genes.

The present invention also includes synthetic peptides made using the nucleotide sequence information comprising cDNA or genomic clone embodiments of the invention. The invention includes either naturally occurring or synthetic peptides which may be used as antigens for the production of MSH^R-specific antibodies, or used for competitors of the MSH^R molecule for drug binding, or to be used for the production of inhibitors of the binding of agonists or antagonists or analogues thereof to MSH^R molecule.

The present invention also provides antibodies against and epitopes of mammalian MSH^Rs, preferably mouse or human MSH^R proteins. It is an object of the present invention to provide antibodies that are immunologically reactive to a mammalian MSH^R protein. It is a particular object of the invention to provide a monoclonal antibodies to mammalian MSH^R protein, most preferably mouse or

human MSH^R protein.

It is also an object of the present invention to provide a hybridoma cell line that produces such an antibody. It is a particular object of the invention to provide a hybridoma cell line that is the result of fusion between a non-immunoglobulin producing mouse myeloma cell line and spleen cells derived from a mouse immunized with a human cell line which expresses MSH^R antigen. The present invention also provides a hybridoma cell line that produces such an antibody, and that can be injected into a living mouse to provide an ascites fluid from the mouse that is comprised of such an antibody.

10 The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal antibody that is immunologically reactive to a mammalian MSH^R, preferably a mouse or human MSH^R, and in a pharmaceutically acceptable carrier.

15 It is a further object of the present invention to provide an epitope of a mammalian MSH^R protein wherein the epitope is immunologically reactive to an antibody specific for the mammalian MSH^R. In preferred embodiments, the epitope is derived from mouse or human MSH^R protein.

20 It is another object of the invention to provide a chimeric antibody that is immunologically reactive to a mammalian MSH^R protein. In a preferred embodiment, the chimeric antibody is a monoclonal antibody. In a preferred embodiment, the MSH^R is a mouse or human MSH^R.

25 The present invention provides a recombinant expression construct comprising the nucleotide sequence of a mammalian MSH^R, preferably the mouse or human MSH^R and sequences sufficient to direct the synthesis of mouse or human MSH^R in cultures of transformed eukaryotic cells. In a preferred embodiment, the recombinant expression construct is comprised of plasmid sequences derived from the plasmid pcDNA1/neo and cDNA or genomic DNA of mouse or human MSH^R gene. This invention includes a recombinant expression construct comprising essentially the nucleotide sequences of genomic or cDNA clones of mouse or human MSH^R in an embodiment that provides for their expression in cultures of transformed eukaryotic cells.

5 It is also an object of this invention to provide cultures of transformed eukaryotic cells that have been transformed with such a recombinant expression construct and that synthesize mammalian, preferably mouse or human, MSH^R protein. In a preferred embodiment, the invention provides human 293 cells that synthesize mouse MSH^R. In an additional preferred embodiment, the invention provides human 293 cells that synthesize human MSH^R protein.

10 The present invention also includes protein preparations of mammalian, preferably mouse or human MSH^R, and preparations of membranes containing mammalian MSH^R, derived from cultures of transformed eukaryotic cells. In a preferred embodiment, cell membranes containing mouse MSH^R protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs the synthesis of mouse MSH^R. In another preferred embodiment, cell membranes containing human MSH^R protein are isolated from 293 cell cultures transformed with a recombinant expression construct that directs 15 the synthesis of human MSH^R. It also an object of this invention to provide mammalian, preferably mouse or human MSH^R for use in the *in vitro* screening of novel adenosine agonist and antagonist compounds. In a preferred embodiment, membrane preparations containing the mouse MSH^R, derived from cultures of transformed eukaryotic cells, are used to determine the drug 20 dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. In another preferred embodiment, membrane preparations containing the human MSH^R, derived from cultures of transformed eukaryotic cells, are used to determine the drug dissociation properties of various novel adenosine agonist and antagonist compounds *in vitro*. These properties are then 25 used to characterize such novel compounds by comparison to the binding properties of known mouse or human MSH^R agonists and antagonists.

The present invention will also be useful for the *in vivo* detection of analogues of agonists or antagonists of MSH^R, known or unknown, either naturally occurring or as the embodiments of a drug.

30 It is an object of the present invention to provide a method for the quantitative detection of agonists or antagonists, or analogues thereof, of MSH^R,

known or unknown, either naturally occurring or as the embodiments of a drug. It is an additional object of the invention to provide a method to detect such agonists, antagonists, or analogues thereof in blood, saliva, semen, cerebrospinal fluid, plasma, lymph, or any other bodily fluid.

5 Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 The term "melanocyte stimulating hormone receptor" as used herein refers to proteins substantially homologous to, and having substantially the same biological activity as, the protein coded for by the nucleotide sequence depicted in Figure 1 (SEQ ID NO:3). This definition is intended to encompass natural allelic variations in the melanocyte stimulating hormone receptor sequence. Cloned genes of the present invention may code for MSH^Rs of any species of origin, including, for example, mouse, rat, rabbit, cat, and human, but preferably code for receptors of mammalian, most preferably mouse and human, origin.

10 Nucleic acid hybridization probes provided by the invention comprise DNA sequences that are substantially homologous to the DNA sequences in Figure 1A (SEQ ID NO:3) and 1B (SEQ ID NO:5). Nucleic acid probes are useful for detecting MSH^R gene expression in cells and tissues using techniques well-known in the art, including but not limited to Northern blot hybridization, 15 *in situ* hybridization and Southern hybridization to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotides probes derived therefrom, are useful are also useful for Southern hybridization of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) 20 associated with certain genetic disorders.

25 The production of proteins such as the MSH^R from cloned genes by genetic engineering is well known. See, e.g., U.S. Patent No. 4,761,371 to Bell *et al.* at Col. 6 line 3 to Col. 9 line 65. (The disclosure of all U.S. patent references cited herein is to be incorporated herein by reference.) The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

30 DNA which encodes the MSH^R may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide

probes generated from the MSH^R gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, MSH^R gene sequences may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the MSH^R gene sequence provided herein. See U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis.

The MSH^R may be synthesized in host cells transformed with a recombinant expression construct comprising a DNA sequence encoding the MSH^R. Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the MSH^R and/or to express DNA which encodes the MSH^R. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a DNA sequence encoding the MSH^R is operably linked to suitable control sequences capable of effecting the expression of the MSH^R in a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (i.e., fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the

intended expression host. A preferred vector is the plasmid pcDNA1/neo. Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising a mammalian MSH^R. Transformed host cells may ordinarily express the mammalian MSH^R, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor. When expressed, the mammalian MSH^R will typically be located in the host cell membrane.

DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leaders sequences, contiguous and in the same translational reading frame.

Cultures of cells derived from multicellular organisms are a desirable host for recombinant MSH^R synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. See Tissue Culture, Academic Press, Kruse & Patterson, editors (1973). Examples of useful host cell lines are human 293 cells, VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and WI38, BHK, COS-7, CV, and MDCK cell lines. Human 293 cells are preferred. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the gene to be expressed, along with a ribosome binding site, RNA splice sites (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g., polyoma, adenovirus, VSV, or MPV), or may be provided by the host cell chromosomal replication mechanism. If the vector is

integrated into the host cell chromosome, the latter may be sufficient.

The invention provides homogeneous compositions of mammalian MSH^R protein produced by transformed eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian MSH^R protein that comprises 90% of the protein in such homogenous composition.

5 Mammalian MSH^R protein made from cloned genes in accordance with the present invention may be used for screening agonist compounds for MSH^R activity, or for determining the amount of a MSH^R agonist or antagonist drug in a solution (e.g., blood plasma or serum). For example, host cells may be
10 transformed with a recombinant expression construct of the present invention, MSH^R expressed in that host, the cells lysed, and the membranes from those cells used to screen compounds for MSH^R binding activity. Competitive binding assays in which such procedures may be carried out are well known in the art. By selection of host cells which do not ordinarily express MSH^Rs, pure preparations
15 of membranes containing MSH^Rs can be obtained. Further, MSH^R agonists and antagonists can be identified by transforming host cells with vectors of the present invention. Membranes obtained from such cells can be used in binding studies wherein the drug dissociation activity is monitored.

20 The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express the MSH^R to thereafter express this receptor. Such cells are useful as intermediates for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Further, genes and vectors comprising the recombinant expression construct of the present invention are useful in gene
25 therapy. For such purposes, retroviral vectors as described in U.S. Patent No. 4,650,764 to Temin & Watanabe or U.S. Patent No. 4,861,719 to Miller may be employed. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out homologous recombination or site-directed mutagenesis. *See generally* Thomas & Capecchi, 1987, *Cell* 51: 503-512; Bertling, 1987, *Bioscience Reports* 7: 107-112; Smithies *et al.*, 1985, *Nature* 317: 30
30 230-234.

5 Oligonucleotides of the present invention are useful as diagnostic tools for probing MSH receptor gene expression in tissues. For example, tissues can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the MSH^R gene, and potential pathological conditions related thereto, as also illustrated by the Examples below.

10 The invention also provides antibodies that are immunologically reactive to a mammalian MSH^R. The antibodies provided by the invention can be raised in animals by inoculation with cells that express a mammalian MSH^R or epitopes of a mammalian MSH^R using methods well known in the art. Animals that can be used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals 15 for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

20 Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell line which naturally expresses a mammalian MSH^R, or any cell or cell line that expresses a mammalian MSH^R or any epitope therein as a result of molecular or genetic engineering, or that has been treated to increase the expression of a mammalian MSH^R by physical, biochemical or genetic means. Preferred cells are human cells, most preferably human 293 cells that have been transformed with a recombinant expression construct comprising DNA sequences encoding a mammalian MSH^R and that express the mammalian MSH^R gene product.

25 The present invention provides monoclonal antibodies that are immunologically reactive with an epitope that is a mammalian MSH^R present on the surface of mammalian cells, preferably human or mouse cells. These antibodies are made using methods and techniques well known to those of skill in the art.

30 Monoclonal antibodies provided by the present invention are produced by

hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with cells expressing a mammalian MSH^R, including human cells, as 5 described above. The myeloma cell lines used in the invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from mouse, and the most preferred mouse myeloma cell line is P3X63-Ag8.653. The animals from whom spleens are obtained after 10 immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence 15 of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention can also be 20 produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a mammalian MSH^R.

The present invention encompasses fragments of the antibody that are 25 immunologically reactive with an epitope of a mammalian MSH^R. Such fragments can be produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian MSH^R made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mammalian MSH^R 30 that is comprised of sequences and/or a conformation of sequences present in the

5 mammalian MSH^R molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian MSH^R molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art.

10 The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

15 The invention also includes chimeric antibodies, comprised of immunologically reactive light chain and heavy chain peptides to an epitope that is a mammalian MSH^R. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

20 The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

25 **Isolation of an α MSH Receptor Probe by Random PCR Amplification of Human Melanoma cDNA Using Degenerate Oligonucleotide Primers**

30 In order to clone novel G-protein coupled receptors, human melanoma cDNA was used as template for a polymerase chain reaction (PCR)-based random cloning experiment. PCR was performed using a pair of degenerate oligonucleotide primers corresponding to the putative third and sixth transmembrane regions of G-protein coupled receptors (Libert *et al.*, 1989, *Science* **244**: 569-72; Zhou *et al.*, 1990, *Nature* **347**: 76-80). The PCR products obtained in this experiment were characterized by nucleotide sequencing. Two novel sequences representing novel G-protein-coupled receptors were identified.

35 PCR amplification was performed as follows. Total RNA was isolated from a human melanoma tumor sample by the guanidinium thiocyanate method

(Chirgwin *et al.*, 1979, *Biochemistry* **18**: 5294-5299). Double-stranded cDNA was synthesized from total RNA with murine reverse transcriptase (BRL, Gaithersburg, MD) by oligo-dT priming [Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), 1990]. The melanoma cDNA mixture was then subjected to 45 cycles of PCR amplification using 500 picomoles of degenerate oligonucleotide primers having the following sequence:

10 Primer III (sense):

10 GAGTCGACCTGTG(C/T)G(C/T)(C/G)AT(C/T)(A/G)C₁T(G/T)GAC(C/A)G(C/G)T
AC
(SEQ ID NO:1)

15 and

Primer VI (antisense):

20 CAGAATTTCAG(T/A)AGGGCAICCAGCAGAI(G/C)(G/A)(T/C)GAA
(SEQ ID NO:2)
in 100 μ l of a solution containing 50 mM Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 0.01% gelatin, 200 μ M each dNTP, and 2.5 units of *Taq* polymerase (Saiki *et al.*, 1988, *Science* **239**: 487-491). These primers were commercially synthesized by Research Genetics Inc. (Huntsville, AL). Each PCR amplification cycle consisted of incubations at 94°C for 1 min (denaturation), 45°C for 2 min (annealing), and 25 72°C for 2 min (extension).

30 Amplified products of the PCR reaction were extracted with phenol/chloroform and precipitated with ethanol. After digestion with *Eco*RI and *Sal*I, the PCR products were separated on a 1.2% agarose gel. A slice of this gel, corresponding to PCR products of 300 basepairs (bp) in size, was cut out and purified using glass beads and sodium iodide, and the insert was then cloned into a pBKS cloning vector (Stratagene, LaJolla, CA).

35 A total of 172 of such pBKS clones containing inserts were sequenced using Sequenase (U. S. Biochemical Corp., Cleveland, OH) by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977, *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467). Two types of sequences homologous to other

G-protein coupled receptors were identified.

EXAMPLE 2

5

Isolation and Sequence Analysis of Mouse α MSH Receptor cDNA

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Probes isolated in Example 1 was used to screen a Cloudman melanoma cDNA library in order to isolate a full-length cDNA corresponding to the cloned probe. One clone was isolated from a library of 5×10^6 clones screened as described below. This clone contained an insert of 2.6 kilobases (kb). The nucleotide sequence of the complete coding region was determined, as shown in Figure 1A (SEQ ID NO:3).

15

The PCR probe was labeled by the random-priming method (Stratagene PrimeIt, #300387, LaJolla, CA) and used to screen a Cloudman melanoma line cDNA library constructed in the λ ZAP vector (Stratagene). Library screening was performed using techniques well-known in the art as described in Bunzow *et al.* (1988, *Nature* **336**: 783-787) at moderate stringency (40% formamide, 1M NaCl, 50mM Tris-HCl, pH 7.5, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 100 μ g/ml salmon sperm DNA, 10X Denhardt's solution). One cDNA clone was identified (termed mmelA) and its 2.6 kb cDNA insert was isolated and subcloned into pBKS (Stratagene); the resulting plasmid was called pmmelA. Nucleotide sequence analysis and homology comparisons were done on the OHSU computer system with software provided by Intelligenetics Inc. (Mountain View, CA).

20

The nucleotide sequence of pmmelA (the cDNA clone isolated as described above) is shown in Figure 1A (SEQ ID NO:3). The longest open reading frame of this cDNA encodes a predicted protein product of 315 amino acids with a calculated molecular weight of 35.3 kilodaltons (kD). The deduced amino acid sequence is shown in Figure 2 (SEQ ID NO:4) as mouse MSH-R. Single letter amino acid codes are used [see, G. Zubay, *Biochemistry* (2d ed.), 1988 (MacMillen Publishing: New York) p.33]. Uppercase lettering indicates amino acid residues in common between the receptor proteins shown; lowercase lettering indicates divergent residues.

Hydrophobicity analysis (Kyte & Doolittle, 1982, *J. Mol. Biol.* 157: 105-132) of the deduced amino acid sequence showed that the protein contains seven hydrophobic stretches of 21 to 26 amino acids apiece. Putative transmembrane domains are overlined and designated with Roman numerals.

5

EXAMPLE 3

Construction of Mouse α MSH^R Expression Plasmids, DNA Transfection and Functional Expression of the α MSH^R Gene Product

10 In order to biochemically characterize the putative mouse α MSH^R cDNA isolated as in Example 2, and to confirm that it encodes an α MSH receptor, mmelA was cloned into a mammalian expression vector, this vector transfected into human 293 cells, and cell lines generated that expressed the putative α MSH^R receptor at the cell surface. Such cells and membranes isolated from such cells 15 were used for biochemical characterization experiments described below.

20 The entire coding region of the α MSH^R cDNA insert from mmelA contained in a 2.1kb fragment was excised from pBSK and subcloned into the *Bam*HI/*Xho*I sites of pcDNAI/neo expression vector (Invitrogen, San Diego, CA). The resulting plasmid was called pcDNA-mmelA. pcDNA-mmelA plasmid DNA 25 was prepared in large-scale through one cycle of CsCl gradient ultracentrifugation and 20 μ g pcDNA-mmelA DNA were transfected into each 100mm dish of 293 cells using the calcium phosphate method (see Chen & Okayama, 1987, *Mol. Cell. Biol.* 7: 2745-2752). After transfection, cells were cultured in DMEM media supplemented with 10% calf serum in a 3% CO₂ atmosphere at 37°C. Selection was performed with neomycin (G418; GIBCO) at a concentration of 1000 μ g/ml; selection was started 72 hr after transfection and continued for 3 weeks.

30 The α MSH^R is known to couple to G-proteins and thereby activate adenylyl cyclase, increasing intracellular levels of cAMP (see Buckley & Ramachandran, 1981, *Proc. Natl. Acad. Sci. USA* 78: 7431-7435; Grahame-Smith *et al.*, 1967, *J. Biol. Chem.* 242: 5535-5541; Mertz & Catt, 1991, *Proc. Natl. Acad. Sci. USA* 88: 8525-8529; Pawalek *et al.*, 1976, *Invest. Dermatol.* 66: 200-209). This

property of cells expressing the α MSH receptor was used to analyze expression of the α MSH receptor in cell colonies transfected with the expression vectors described herein as follows. Cells ($\sim 1 \times 10^6$) were plated in 6-well dishes, washed once with DMEM containing 1% bovine serum albumin (BSA) and 0.5mM IBMX (a phosphodiesterase inhibitor), then incubated for 45 minutes at 37°C with varying concentrations of the melanotropic peptides α MSH, β MSH, γ MSH, the MSH peptide analogues Nle⁴, D-Phe⁷- α MSH (NDP-MSH), and ACTH. Following hormone treatment, the cells were washed twice with phosphate buffered saline and intracellular cAMP extracted by lysing the cells with 1ml of 60% ethanol. Intracellular cAMP concentrations were determined using an assay (Amersham) which measures the ability of cAMP to displace [8^3 H] cAMP from a high affinity cAMP binding protein (see Gilman, 1970, Proc. Natl. Acad. Sci. USA 67: 305-312).

The results of these experiments are shown in Figure 3. The abscissa indicates the concentration of each hormone and the ordinate indicates the percentage of basal intracellular cAMP concentration achieved by each treatment. Points indicate the mean of duplicate incubations; the standard error did not exceed 15% for any data point. None of the peptides tested induced any change in intracellular cAMP in cells containing the vector alone. Cells expressing the murine α MSH receptor responded to melanotropic peptides with a 2-3 fold elevation of intracellular cAMP, similar to levels of cAMP induced by these peptides in the Cloudman cell line (see Pawalek, 1985, Yale J. Biol. Med. 58: 571-578). The EC₅₀ values determined for α MSH (2.0×10^{-9} M), ACTH (8.0×10^{-9} M) and the superpotent MSH analogue NDP-MSH (2.8×10^{-11} M) correspond closely to reported values (see Tatro *et al.*, 1990, Cancer Res. 50: 1237-1242). As expected, the β MSH peptide had an EC₅₀ value comparable to α MSH²² while γ MSH had little or no activity (see Slominski *et al.*, 1992, Life Sci. 50: 1103-1108), confirming the identity of this receptor as a melanocyte α MSH receptor.

EXAMPLE 4

Isolation and Characterization of a Human α MSH^R Genomic Clone

5 In order to isolate a human counterpart of the murine melanocyte α MSH receptor gene, a human genomic library was screened at high stringency (50% formamide, 42°C) using the human PCR fragments isolated as described in Example 1. Two different types of sequences were isolated, corresponding to the two PCR fragments, and were found to encode highly related G protein-coupled
10 receptors. These genomic clones were sequenced as described in Example 2. One of these genomic clones was determined to encode an human MSH receptor (SEQ ID NO:5). The human MSH receptor has a predicted amino acid sequence (SEQ ID NO:6) that is 75% identical and colinear with the mouse α MSH receptor cDNA sequence (Figure 2), represented as human MSH-R. The predicted
15 molecular weight of the human MSH^R is 34.7kD.

20 The predicted amino acid sequences of the mouse α MSH^R (SEQ ID NO:4) and human MSH^R (SEQ ID NO:6) are aligned in Figure 2. These sequences define the melanocortin receptors as a novel subfamily of the G protein-coupled receptors with a number of unusual features. The melanocortin receptors are the smallest G protein-coupled receptors identified to date (297-317aa) resulting from a short amino terminal extracellular domain, a short carboxy-terminal intracellular domain, and a very small third intracellular loop. The melanocortin receptors are lack several amino acid residues present in most G protein coupled receptors (see Probst *et al.*, 1992, *DNA & Cell Biol.* **11**: 1-20), including the proline residues
25 in the 4th and 5th transmembrane domains, likely to introduce a bend in the alpha helical structure of the transmembrane domains and thought to be involved in the formation of the binding pocket (see Applebury & Hargrave, 1986, *Vision Res.* **26**: 1881-1895), and one or both of the cysteine residues thought to form a disulfide bond between the first and second extracellular loops (see Dixon *et al.*, 1987, *EMBO J.* **6**: 3269-3275 and Karnik *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* **85**: 8459-8463). Remarkably, the melanocortin receptors do not appear highly related to the other G protein-coupled receptors which recognize peptide ligands, such as the receptors for bombesin (see Spindel *et al.*, 1990, *Mol.*

5 Endocrinol. 4: 1956-1963) or substance K (see Masu *et al.*, 1987, Nature 329: 836-838) but rather, are more closely related to the receptor for Δ^9 -tetrahydrocannabinol (see Matsuda *et al.*, 1990, Nature 346: 561-564). The cannabinoid receptor also lacks the conserved proline in transmembrane 5 and the cysteine in the first extracellular loop necessary for disulfide bond formation. Least parsimony analysis with the receptor sequences shown in Figure 2 suggests the cannabinoid and melanocortin receptors may be evolutionarily related and form a subfamily distinct from the peptide receptors and the amine receptors. 10 Regardless of whether the similarities are the result of evolutionary conservation or convergence, the sequence and putative structural similarities between the melanocortin and cannabinoid receptors may be informative in the search for the endogenous cannabinoid-like ligand.

EXAMPLE 5

15

Tissue Distribution of α MSH Receptors

To further gain insight into these receptors, we have examined the tissue distribution of their corresponding mRNAs from various tissues by performing Northern hybridization experiments on RNA isolated from various tissues (see Maniatis *et al.*, *ibid.*). The results of these experiments are shown in Figure 4.

25 A panel of tissue samples was examined by Northern hybridization analysis performed under high stringency conditions. The same nitrocellulose filter was hybridized successively with a human MSH receptor probe and a mouse MSH receptor probe to determine the distribution of each receptor mRNA. The murine MSH receptor is encoded predominantly by a single mRNA species of 3.9kb, while the human MSH receptor is encoded, in two melanoma samples, predominantly by a 3.0kb species. High levels of receptor mRNA are seen in both primary mouse melanocytes and mouse melanoma cell lines. In contrast, 30 extremely low levels of receptor mRNA were detected in primary human melanocytes, and many human melanoma samples (see melanoma 1, Fig. 4). Most intriguing is the dramatic elevation of MSH-R mRNA seen thus far in 3 of 11 samples tested, such as is seen in melanoma sample #2 (Fig. 4).

Additionally, we have been unable to detect expression in the brain of any of the receptors described here, despite extensive documentation of MSH binding sites there as well as in other tissues. These finding suggest the existence of alternate forms of these or related receptors that may be specifically expressed in
5 brain tissue.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cone, Roger D
Mountjoy, Kathleen G

(ii) TITLE OF INVENTION: Melanocyte Stimulating Hormone Receptor
and Uses

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: USA
(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US93/03247
(B) FILING DATE: 07-APR-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Noonan, Kevin E
(B) REGISTRATION NUMBER: 35,303
(C) REFERENCE/DOCKET NUMBER: 92,154-A

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 312-715-1234
(C) TELEX: 910-221-5317

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature

-25-

- (B) LOCATION: 1..33
- (D) OTHER INFORMATION: /function= "Degenerate oligonucleotide primer (sense)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGTCGACCT GTGYGYSATY RCTKGACMGS TAC

33

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..31
- (D) OTHER INFORMATION: /function= "Degenerate oligonucleotide primer (antisense)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGAAATTCAAG WAGGGCACCA GCAGASRYGA A

31

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1260 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 15..959

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..14

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 960..1260

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Asn	Ala	Thr	Ser	His	Leu	Gly	Leu	Ala	Thr	Asn	Gln	Ser	Glu	Pro	Trp
20															30
Cys	Leu	Tyr	Val	Ser	Ile	Pro	Asp	Gly	Leu	Phe	Leu	Ser	Leu	Gly	Leu
35															45
Val	Ser	Leu	Val	Glu	Asn	Val	Leu	Val	Val	Ile	Ala	Ile	Thr	Lys	Asn
50															60
Arg	Asn	Leu	His	Ser	Pro	Met	Tyr	Tyr	Phe	Ile	Cys	Cys	Leu	Ala	Leu
65															80
Ser	Asp	Leu	Met	Val	Ser	Val	Ser	Ile	Val	Leu	Glu	Thr	Thr	Ile	Ile
85															95
Leu	Leu	Leu	Glu	Val	Gly	Ile	Leu	Val	Ala	Arg	Val	Ala	Leu	Val	Gln
100															110
Gln	Leu	Asp	Asn	Leu	Ile	Asp	Val	Leu	Ile	Cys	Gly	Ser	Met	Val	Ser
115															125
Ser	Leu	Cys	Phe	Leu	Gly	Ile	Ile	Ala	Ile	Asp	Arg	Tyr	Ile	Ser	Ile
130															140
Phe	Tyr	Ala	Leu	Arg	Tyr	His	Ser	Ile	Val	Thr	Leu	Pro	Arg	Ala	Arg
145															160
Arg	Ala	Val	Val	Gly	Ile	Trp	Met	Val	Ser	Ile	Val	Ser	Ser	Thr	Leu
165															175
Phe	Ile	Thr	Tyr	Tyr	Lys	His	Thr	Ala	Val	Leu	Leu	Cys	Leu	Val	Thr
180															190
Phe	Phe	Leu	Ala	Met	Leu	Ala	Leu	Met	Ala	Ile	Leu	Tyr	Ala	His	Met
195															205
Phe	Thr	Arg	Ala	Cys	Gln	His	Val	Gln	Gly	Ile	Ala	Gln	Leu	His	Lys
210															220
Arg	Arg	Arg	Ser	Ile	Arg	Gln	Gly	Phe	Cys	Leu	Lys	Gly	Ala	Ala	Thr
225															240
Leu	Thr	Ile	Leu	Leu	Gly	Ile	Phe	Phe	Leu	Cys	Trp	Gly	Pro	Phe	Phe
245															255
Leu	His	Leu	Leu	Leu	Ile	Val	Leu	Cys	Pro	Gln	His	Pro	Thr	Cys	Ser
260															270
Cys	Ile	Phe	Lys	Asn	Phe	Asn	Leu	Phe	Leu	Leu	Ile	Val	Leu	Ser	
275															285
Ser	Thr	Val	Asp	Pro	Leu	Ile	Tyr	Ala	Phe	Arg	Ser	Gln	Glu	Leu	Arg
290															300

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Met Thr Leu Lys Glu Val Leu Leu Cys Ser Trp
 305 310 315

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1633 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 462..1415

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR
- (B) LOCATION: 1..461

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 1416..1633

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCGCATGTG	GCCGCCCTCA	ATGGAGGGCT	CTGAGAACGA	CITTTAAAC	GCAGAGAAAA	60
AGCTCCATTC	TTCCCAGACC	TCAGCGCAGC	CCTGGCCCAG	GAAGGGAGGA	GACAGAGGCC	120
AGGACGGTCC	AGAGGTGTCG	AAATGTCCTG	GGAACCTGAG	CAGCAGCCAC	CAGGGAAGAG	180
GCACGGGAGGG	AGCTGAGGAC	CAGGCTTGGT	TGTGAGAATC	CCTGAGCCCA	GGCGGTTGAT	240
GCCAGGGAGGT	GTCTGGACTG	GCTGGGCCAT	GCCTGGGCTG	ACCTGTCCAG	CCAGGGAGAG	300
GGTGTGAGGG	CAGATCTGGG	GGTGCCCAGA	TGGAAGGAGG	CAGGCATGGG	GACACCCAAG	360
GCCCCCTGGC	AGCACCATGA	ACTAACGAGG	ACACCTGGAG	GGGAAGAACT	GTGGGGACCT	420
GGAGGGCTCC	AACGACTCCT	TCCTGCTTCC	TGGACAGGAC	T ATG GCT GTG CAG		473
				Met Ala Val Gln		
				1		
GGA TCC CAG AGA AGA	CTT CTG	GGC TCC	CTC AAC	TCC ACC CCC	ACA GCC	521
Gly Ser Gln Arg Arg	Leu Leu	Gly Ser	Leu Asn	Ser Thr	Pro Thr Ala	
5	10	15	20			
ATC CCC CAG CTG GGG	CTG GCT	GCC AAC	CAG ACA	GGA GCC	CGG TGC CTG	569
Ile Pro Gln Leu	Gly Leu	Ala Ala	Asn Gln	Thr Gly	Ala Arg Cys Leu	
25	30	35				

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GAG GTG TCC ATC TCT GAC GGG CTC TTC CTC AGC CTG CGG CTG GTG AGC Glu Val Ser Ile Ser Asp Gly Leu Phe Leu Ser Leu Gly Leu Val Ser 40 45 50	617
TTG GTG GAG AAC GCG CTG GTG GTG GCC ACC ATC GCC AAG AAC CGG AAC Leu Val Glu Asn Ala Leu Val Val Ala Thr Ile Ala Lys Asn Arg Asn 55 60 65	665
CTG CAC TCA CCC ATG TAC TGC TTC ATC TGC TGC CTG GCC TTG TCG GAC Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu Ala Leu Ser Asp 70 75 80	713
CTG CTG GTG AGC GGG ACG AAC GTG CTG GAG ACG GCC GTC ATC CTC CTG Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala Val Ile Leu Leu 85 90 95 100	761
CTG GAG GCC GGT GCA CTG GTG GCC CGG GCT GCG GTG CTG CAG CAG CTG Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val Leu Gln Gln Leu 105 110 115	809
GAC AAT GTC ATT GAC GTG ATC ACC TGC AGC TCC ATG CTG TCC AGC CTC Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met Leu Ser Ser Leu 120 125 130	857
TGC TTC CTG GGC GCC ATC GCC GTG GAC CGC TAC ATC TCC ATC TTC TAC Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile Ser Ile Phe Tyr 135 140 145	905
GCA CTG CGC TAC CAC AGC ATC GTG ACC CTG CCG CCG GCG CCG CGA GCC Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg Ala Pro Arg Ala 150 155 160	953
GTT GCG GCC ATC TGG GTG GCC AGT GTC GTC TTC ACC ACG CTC TTC ATC Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser Thr Leu Phe Ile 165 170 175 180	1001
GCC TAC TAC GAC CAC GTG GCC GTC CTG CTG TGC CTC GTG GTC TTC TTC Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu Val Val Phe Phe 185 190 195	1049
CTG GCT ATG CTG GTG CTC ATG GCC GTG CTG TAC GTC CAC ATG CTG GCC Leu Ala Met Leu Val Met Ala Val Leu Tyr Val His Met Leu Ala 200 205 210	1097
CGG GCC TGC CAG CAC GCC CAG GGC ATC GCC CGG CTC CAC AAG AGG CAG Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu His Lys Arg Gln 215 220 225	1145
CGC CCG GTC CAC CAG GGC TTT GGC CTT AAA GGC GCT GTC ACC CTC ACC Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala Val Thr Leu Thr 230 235 240	1193

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ATC CTG CTG GGC ATT TTC TTC CTC TGC TGG GGC CCC TTC TTC CTG CAT Ile Leu Leu Gly Ile Phe Phe Leu Cys Trp Gly Pro Phe Phe Leu His 245 250 255 260	1241
CTC ACA CTC ATC GTC CTC TGC CCC GAG CAC CCC ACG TGC GGC TGC ATC Leu Thr Leu Ile Val Leu Cys Pro Glu His Pro Thr Cys Gly Cys Ile 265 270 275	1289
TTC AAG AAC TTC AAC CTC TTT CTC GCC CTC ATC ATC TGC AAT GCC ATC Phe Lys Asn Phe Asn Leu Phe Leu Ala Leu Ile Ile Cys Asn Ala Ile 280 285 290	1337
ATC GAC CCC CTC ATC TAC GCC TTC CAC AGC CAG GAG CTC CGC AGG ACG Ile Asp Pro Leu Ile Tyr Ala Phe His Ser Gln Glu Leu Arg Arg Thr 295 300 305	1385
CTC AAG GAG GTG CTG ACA TGC TCC TGG TGAGCGCGGT GCACGCGCTT Leu Lys Glu Val Leu Thr Cys Ser Trp 310 315	1432
TAAGTGTGCT GGGCAGAGGG AGGTGGTGAT ATTGTGGTCT GGTTCTGTG TGACCCCTGGG	1492
CAGTTCCCTTA CCTCCCTGGT CCCCCTTTGT CAAAGAGGAT GGACTAAATG ATCTCTGAAA	1552
GTGTTGAAGC GCGGACCCCTT CTGGGCAGGG AGGGGTCCCTG CAAAATCCA GGCAAGGACTT	1612
CTCACCAAGCA GTCGTGGGAA C	1633

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 317 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Val Gln Gly Ser Gln Arg Arg Leu Leu Gly Ser Leu Asn Ser 1 5 10 15
Thr Pro Thr Ala Ile Pro Gln Leu Gly Leu Ala Ala Asn Gln Thr Gly 20 25 30
Ala Arg Cys Leu Glu Val Ser Ile Ser Asp Gly Leu Phe Leu Ser Leu 35 40 45
Gly Leu Val Ser Leu Val Glu Asn Ala Leu Val Val Ala Thr Ile Ala 50 55 60
Lys Asn Arg Asn Leu His Ser Pro Met Tyr Cys Phe Ile Cys Cys Leu 65 70 75 80

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Ala Leu Ser Asp Leu Leu Val Ser Gly Thr Asn Val Leu Glu Thr Ala
85 90 95

Val Ile Leu Leu Leu Glu Ala Gly Ala Leu Val Ala Arg Ala Ala Val
100 105 110

Leu Gln Gln Leu Asp Asn Val Ile Asp Val Ile Thr Cys Ser Ser Met
115 120 125

Leu Ser Ser Leu Cys Phe Leu Gly Ala Ile Ala Val Asp Arg Tyr Ile
130 135 140

Ser Ile Phe Tyr Ala Leu Arg Tyr His Ser Ile Val Thr Leu Pro Arg
145 150 155 160

Ala Pro Arg Ala Val Ala Ala Ile Trp Val Ala Ser Val Val Phe Ser
165 170 175

Thr Leu Phe Ile Ala Tyr Tyr Asp His Val Ala Val Leu Leu Cys Leu
180 185 190

Val Val Phe Phe Leu Ala Met Leu Val Leu Met Ala Val Leu Tyr Val
195 200 205

His Met Leu Ala Arg Ala Cys Gln His Ala Gln Gly Ile Ala Arg Leu
210 215 220

His Lys Arg Gln Arg Pro Val His Gln Gly Phe Gly Leu Lys Gly Ala
225 230 235 240

Val Thr Leu Thr Ile Leu Leu Gly Ile Phe Phe Leu Cys Trp Gly Pro
245 250 255

Phe Phe Leu His Leu Thr Leu Ile Val Leu Cys Pro Glu His Pro Thr
260 265 270

Cys Gly Cys Ile Phe Lys Asn Phe Asn Leu Phe Leu Ala Leu Ile Ile
275 280 285

Cys Asn Ala Ile Ile Asp Pro Leu Ile Tyr Ala Phe His Ser Gln Glu
290 295 300

Leu Arg Arg Thr Leu Lys Glu Val Leu Thr Cys Ser Trp
305 310 315

SUBSTITUTE SHEET

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WHAT WE CLAIM IS:

1. A nucleic acid comprising a nucleotide sequence encoding a mammalian melanocyte stimulating hormone receptor.
2. A nucleic acid according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.
3. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1A (SEQ ID NO:3).
4. A nucleic acid according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.
105. A nucleic acid according to Claim 1 wherein the nucleotide sequence is substantially homologous to the sequence in Figure 1B (SEQ ID NO:5).
6. A DNA sequence according to Claim 1 wherein the mammalian melanocyte stimulating hormone receptor encoded therein has the melanotropic peptide response properties described in Figure 3.
157. A homogeneous composition of a 35.3 kilodalton melanocyte stimulating hormone receptor or derivative thereof, wherein the amino acid sequence of the melanocyte stimulating hormone receptor or derivative thereof comprises the mouse MSH-R sequence shown in Figure 2 (SEQ ID NO:4).
8. A homogeneous composition of a 34.6 kilodalton melanocyte stimulating hormone receptor or derivative thereof, wherein the amino acid sequence of the melanocyte stimulating hormone receptor or derivative thereof comprises the human MSH-R sequence shown in Figure 2 (SEQ ID NO:6).
9. A nucleic acid hybridization probe for the detection of mammalian melanocyte stimulating hormone receptor expression comprising the nucleotide sequence of Claim 3.
10. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a human.
11. The nucleic acid hybridization probe according to Claim 9 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.
12. A nucleic acid hybridization probe for the detection of mammalian melanocyte stimulating hormone receptor expression comprising the nucleotide sequence of Claim 5.
3513. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection and diagnosis of genetic disease in a

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human.

14. The nucleic acid hybridization probe according to Claim 12 whereby the probe is adapted for use in the detection, isolation and characterization of novel mammalian receptor genes.

15. A recombinant expression construct comprising a nucleotide sequence encoding a mammalian melanocyte stimulating hormone receptor.

16. A recombinant expression construct comprising the DNA sequence of Claim 3, wherein the construct is capable of expressing the mouse melanocyte stimulating hormone receptor in a transformed eukaryotic cell culture.

1017. A recombinant expression construct comprising the DNA sequence of Claim 5, wherein the construct is capable of expressing the human melanocyte stimulating hormone receptor in a transformed eukaryotic cell culture.

18. The recombinant expression construct of Claim 15 comprising pcDNA1/neo sequences.

1519. A eukaryotic cell culture transformed with the expression construct of Claim 16, wherein the transformed eukaryotic cell culture is capable of expressing mouse melanocyte stimulating hormone receptor.

20. A eukaryotic cell culture transformed with the expression construct of Claim 17, wherein the transformed eukaryotic cell culture is capable of expressing the human melanocyte stimulating hormone receptor.

21. A method of screening a compound as an inhibitor of agonist binding to a mammalian melanocyte stimulating hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as
25 in Claim 15 capable of expressing the melanocyte stimulating hormone receptor in a eukaryotic cell; and

(b) assaying for ability of the compound to inhibit the binding of a detectable melanocyte stimulating hormone receptor agonist.

22. The method of claim 21 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

23. The method of Claim 21 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

24. A method of quantitatively detecting a compound as an inhibitor of agonist binding to a mammalian melanocyte stimulating hormone receptor, the method comprising the following steps:

(a) transforming a eukaryotic cell culture with an expression construct as

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in Claim 15 capable of expressing the mammalian melanocyte stimulating hormone receptor in a eukaryotic cell; and

(b) assaying for amount of a compound by measuring the extent of inhibition of binding of a detectable receptor agonist.

525. The method of Claim 24 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

26. The method of Claim 24 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

27. The method of Claim 24 wherein the compound to be tested is present in a human.

28. The method of Claim 24 wherein the compound is present in human blood.

29. The method of Claim 24 wherein the compound is present in human cerebrospinal fluid.

30. The method of Claim 24 wherein the compound is unknown.

1531. An antibody or fragment thereof that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.

32. The antibody according to Claim 31, wherein the antibody is a monoclonal antibody.

33. The antibody according to Claim 31, wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

34. The antibody according to Claim 31, wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

35. A cell line which produces an antibody or fragment thereof that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.

2536. The cell line according to Claim 35, wherein the antibody is a monoclonal antibody.

37. The cell line according to Claim 35, wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.

38. The cell line according to Claim 35, wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

39. A pharmaceutical composition comprising a therapeutically effective amount of an antibody or fragment thereof according to claim 31 in a pharmaceutically acceptable carrier.

40. An epitope of a mammalian melanocyte stimulating hormone receptor wherein the epitope is immunologically reactive to the antibody or fragment thereof according to claim 31.

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41. The epitope according to claim 40 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.
42. The epitope according to claim 40 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.
543. A chimeric antibody that is immunologically reactive to a mammalian melanocyte stimulating hormone receptor.
44. The chimeric antibody according to claim 43 wherein the mammalian melanocyte stimulating hormone receptor is the mouse melanocyte stimulating hormone receptor.
1045. The chimeric antibody according to claim 43 wherein the mammalian melanocyte stimulating hormone receptor is the human melanocyte stimulating hormone receptor.

Figure 1A

10 20 30 40 50 60 70
 TTCCCTGACAA GACTATGTCC ACTCAGGAGC CCCAGAAAGAG TCTTCTGGGT TCTCTCAACT CCAATGCCAC
 80 90 100 110 120 130 140
 CTCTCACCTT CGACTGGCCA CCAACCACTC AGAGCCTTGG TCGCTGTATG TGTCCATCCC AGATGGCCTC
 150 160 170 180 190 200 210
 TTCCCTGAGCC TAGGGCTGGT GAGTCTGGTC GAGAAATGTGC TCGTTGTGAT AGCCATCACC AAAAAACCGCA
 220 230 240 250 260 270 280
 ACCTGGCACTC GCCCATGTAT TACTTCATCT CCTGCCCTGGC CCTGTCTGAC CTGATGGTAA GTGTCAGCAT
 290 300 310 320 330 340 350
 CGTGGCTGGAG ACTAATATCA TCCTGCTCTT GGAGGTGGGG ATCCCTGGTGG CCAGAGTGGC TTGGTGCAG
 360 370 380 390 400 410 420
 CAGCTGGACA ACCTCATTGA CGTGGCTCATC TGTGGCTCCA TGGTGTCCAG TCTCTGCTTC CTGGGCATCA
 430 440 450 460 470 480 490
 TTGCTATAGA CCGCTACATC TCCATCTCT ATCCGCTGGC TTATCACAGC ATCGTGACCC TGCCCGAGGC
 500 510 520 530 540 550 560
 ACCACAGGGCT CTGGTGGGCA TCTGGATGGT CAGCATCGTC TCCAGGACCC TCTTATACAC CTACTACAAG
 570 580 590 600 610 620 630
 CACACAGGGCC TTCTGGCTGC CCTCGTCACT TCTCTCTAG CCATGGTGGC ACTCATGGGG ATTCTGTATG
 640 650 660 670 680 690 700
 CCCACATGTT CACCGAGAGGG TGCCAGGACCG TCCAGGGCAT TCCCCAGCTC CACAAAAGCC GGCGGTCCAT
 710 720 730 740 750 760 770
 CGGCCAAGGC TTCTGGCTCA AGGGTGTGGC CACCCCTACT ATCCCTCTGG CGATTTCTT CCTGTGCTGG
 780 790 800 810 820 830 840
 GCCCCCTTCT TCTGGATCT CTGCTCATC GTCTCTGCG CTGAGGACCC CACCTGGCAGC TGGCATCTCA
 850 860 870 880 890 900 910
 AGAACTTCAA CCTCTTCCTC CTCCCTCATCG TCCTCAGCTC CACTGTGAC CCCCTCATCT ATGCTTCCCG
 920 930 940 950 960 970 980
 CAGCCAGGAG CTCCGCATGA CACTCAAGGA CGTCTCTGGT TGCTCCCTGGT GATCAGAGGG CGCTGGCCAG
 990 1000 1010 1020 1030 1040 1050
 AGGCTGACAG TGATATCCAG TGGCCTGGAT CTGAGACCC ACAGGTACTC ATCCCTTCTT GATCTCCATT
 1060 1070 1080 1090 1100 1110 1120
 TGTCTAAGGG TCGACAGGAT GAGCTTAAA ATAGAAACCC AGACTGGCTG GGGCCAGGAG AAAGGCTAAC
 1130 1140 1150 1160 1170 1180 1190
 TGTGACTGCA GGGCTCACCC AGGGCAGCTA CGGGAACTGG AGGAGACAGG GATGGGAACCT GTAGCCCTGA
 1200 1210 1220 1230 1240 1250 1260
 GCAACGGTCA GACCAACAGGC TCCTGAAGAG CTTCACCTCT CCCACCTAC AGGCAACTCC TGCTCAAGCC

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Figure 1B

10	20	30	40	50	60	70
CCCGCATGTG	CCCCCCCTCA	ATGGACGGCT	CTGAGAACGA	CTTTAAAAC	GCAGAGAAAA	ACCTCCATTG
80	90	100	110	120	130	140
TTCCCAGACC	TCAGGGCAGC	CCTGGCCAG	GAAGGCAGGA	GACAGAGGCC	AGGACGGTCC	AGAGGTGTCC
150	160	170	180	190	200	210
AAATGTCCTG	CGAACCTGAG	CAGCAGCCAC	CAGGGAAAGAC	GCAGGGAGGG	ACCTGAGGAC	CAGGCTTGCT
220	230	240	250	260	270	280
TGTGAGAAC	CCTGAGGCCA	GGCGGTTGAT	GCCAGGAGGT	CTCTGGACTG	GCTGGGCCAT	GCCTGGGCTG
290	300	310	320	330	340	350
ACCTGTCAG	CCAGGGAGAG	GGTGTGAGGG	CAGATCTGGG	GGTGGCCAGA	TCGAAGGAGG	CAGGCATGGG
360	370	380	390	400	410	420
GACACCCAAG	CCCCCTGGC	AGCACCATGA	ACTAACCGAG	ACACCTGGAG	GGGAAGAACT	GTGGGGACCT
430	440	450	460	470	480	490
GGAGGGCTCC	AACGACTCCT	TCCTGCTTCC	TGGCAGGAC	TATGGCTGTG	CAGGGATCCC	AGAGAAGACT
500	510	520	530	540	550	560
TCTGGGCTCC	CTCAACTCCA	CCCCCACAGC	CATCCCCAG	CTGGGGCTGG	CTGCCAACCA	GACAGGAGCC
570	580	590	600	610	620	630
CGGTCCCTGG	AGGTGTCAT	CTCTGACGGG	CTCTTCTCA	GCCTGGGGCT	CGTGAGCTTC	GTGGAGAACG
640	650	660	670	680	690	700
CGCTGGTGGT	GGCCACCATC	GCCAAGAAC	GGAACTGCA	CTCACCCATG	TACTGCTTCA	TCTGCTGCCT
710	720	730	740	750	760	770
GGCCTTGTGG	GACCTGCTGG	TGACCGGGAC	GAACCTGCTG	GAGACGGCCG	TCATCCTCCT	GCTGGAGGCC
780	790	800	810	820	830	840
GGTGCAGTC	TGGCCCCGGC	TGCGGTGCTG	CACCACTGCG	ACAATGTCA	TGACGTGATC	ACCTGCAGCT
850	860	870	880	890	900	910
CCATGCTGTC	CACCCCTCTGC	TTCCTGGGG	CCATCCCCGT	GGACCCGTAC	ATCTCCATCT	TCTACGGACT
920	930	940	950	960	970	980
GGCCTACAC	ACCATCGTGA	CCCTGGGGCG	GGCCCCCGGA	GGCGTTGGCG	CCATCTGGGT	GGCCAGTGTG
990	1000	1010	1020	1030	1040	1050
GTCTTCAGCA	CCCTCTTCAT	CCCTACTAC	GACCACTGCG	CCGTCTGCT	GTCCCTCTG	CTCTTCTTCC
1060	1070	1080	1090	1100	1110	1120
TGGCTATGCT	GGTGCTCATG	GGCGTGTGT	ACGTCCACAT	GCTGGGGCGG	GCCTGCCAGG	ACGCCAGGG
1130	1140	1150	1160	1170	1180	1190
CATCCCCCGG	CTCCACAAAGA	GGCACGGCCC	GGTCCACCAAG	GGCTTGGCC	TTAAAGGGCC	TGTCAACCTC
1200	1210	1220	1230	1240	1250	1260
ACCATCCTGC	TGGGCATTTC	CTTCCTCTGC	TGGGGCCCT	TCTTCCTGCA	TCTCACACTC	ATCGCTCTCT

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Figure 1C

1270 1280 1290 1300 1310 1320 1330
CCCCCGACCA CCCCACCGTGC GGCTCCATCT TCAAGAACCTT CAACCTCTTT CTGGCCCTCA TCATCTGCAA
1340 1350 1360 1370 1380 1390 1400
TCCCACATCATC GACCCCCCTCA TCTACCCCTT CCACAGCCAG GAGCTCCGCA GGACGCTCAA GGAGGTGCTG
1410 1420 1430 1440 1450 1460 1470
ACATGCTCCT CCTGAGCCCCG CTGCCACCCGC TTAAAGTGTG CTGGCCAGAG GGAGGTGGTG ATATTGTGGT
1480 1490 1500 1510 1520 1530 1540
CTGGTTCCCTG TGTGACCCCTG CCCACTTCCT TACCTCCCTG GTCCCCGTTT GTCAAAGAGG ATGGACTAAA
1550 1560 1570 1580 1590 1600 1610
TGATCTCTGA AAGTGTGAA CGCCGGACCC TTCTGGCAG GGAGGGTCC TGCAAAACTC CAGCCAGGAC
1620 1630
TTCTCACCAAG CACTCGTGGC AAC

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Figure 2A

mouse MSH-R	m	s	t	Q	e	p	Q	k	a	L	v	G	S	L	N	S	n	a	T	s	h	21	
human MSH-R	m	a	v	Q	g	s	Q	r	r	L	l	G	S	L	N	S	t	p	T	a	i	21	
human ACTH-R																						7	
rat cannab.	m	(2-83)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	102	
																						1	
mouse MSH-R	-	-	-	L	G	L	A	T	N	Q	s	s	p	w	C	L	y	v	S	I	P	D	40
human MSH-R	p	q	L	G	L	A	A	N	N	Q	t	g	a	r	C	L	e	v	S	I	s	D	42
human ACTH-R	y	e	n	i	n	n	T	a	r	n	n	s	d	C	p	r	v	v	I	P	e	28	
rat cannab.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	123	
																						1	
mouse MSH-R	G	L	F	L	S	L	G	L	V	S	L	V	E	N	v	L	V	V	i	A	I	61	
human MSH-R	G	L	F	L	S	L	G	L	V	S	L	V	E	N	a	L	V	V	a	t	I	63	
human ACTH-R	e	i	f	T	i	s	i	v	g	v	l	E	N	l	i	v	v	v	v	v	A	v	7
rat cannab.	-	L	-	L	T	L	G	-	-	V	L	E	N	L	L	V	L	-	-	-	I	142	

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Figure 2B

	II																					
mouse MSH-R	t	K	N	R	N	L	H	c	P	M	Y	F	I	C	C	L	A	L	S	D	82	
human MSH-R	a	K	N	R	N	L	H	s	P	M	Y	c	F	I	C	C	L	A	L	S	D	84
human ACTH-R	f	K	N	k	N	L	q	a	P	M	Y	f	F	I	C	s	L	A	i	S	D	70
rat cannab.	-	-	R	-	L	-	-	P	-	Y	-	F	I	-	S	L	A	-	-	D	163	
	II																			103		
mouse MSH-R	L	m	v	s	v	i	y	L	E	T	t	i	I	L	L	E	v	G	i			
human MSH-R	L	L	v	s	g	t	n	v	L	E	T	a	v	I	L	L	E	a	G	a	105	
human ACTH-R	m	L	g	s	i	y	k	i	L	E	n	i	I	i	L	r	r	m	G	y	91	
rat cannab.	L	L	G	S	V	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	184	
	III																		124			
mouse MSH-R	L	v	A	R	v	A	l	v	Q	Q	L	D	N	I	I	D	Y	I	i	C	g	
human MSH-R	L	v	A	R	a	A	v	l	Q	Q	L	D	N	y	I	D	Y	i	t	C	s	126
human ACTH-R	L	k	p	R	g	s	f	e	t	t	a	D	d	i	I	D	s	i	f	v	i	112
rat cannab.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	v	-	205	

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Figure 2C

	III												IV												
mouse MSH-R	S	M	v	S	S	L	C	F	L	G	i	I	A	i	D	R	Y	I	S	I	F	I	I	145	
human MSH-R	S	M	L	S	S	L	C	F	L	G	a	I	A	v	D	R	Y	I	S	I	F	I	I	147	
human ACTH-R	S	I	L	g	S	i	f	s	L	s	v	I	A	a	D	R	Y	I	S	I	F	I	I	133	
rat cannab.	-	-	-	-	G	S	L	F	-	L	-	Y	-	A	I	D	R	Y	I	S	I	-	-	226	
mouse MSH-R	Y	A	L	R	Y	H	S	I	Y	T	L	P	R	A	v	v	g	I						166	
human MSH-R	Y	A	L	R	Y	H	S	I	Y	T	L	P	R	A	v	a	a	I						168	
human ACTH-R	h	A	L	R	Y	H	S	I	Y	T	m	r	R	t	v	v	I	t	v	I				154	
rat cannab.	-	-	L	-	Y	-	-	I	V	T	-	P	-	A	V	V	A	-	-	-	-	-	-	247	
mouse MSH-R	W	m	v	S	i	Y	s	S	T	L	F	I	t	Y	Y	k	H	t	A	Y	L	I	I	187	
human MSH-R	W	v	a	S	v	V	f	S	T	L	F	I	a	Y	Y	d	H	V	A	V	L	I	I	189	
human ACTH-R	W	T	f	c	t	g	t	g	i	t	m	v	i	f	s	h	H	V	p	t	v	I	I	175	
rat cannab.	W	T	-	-	I	V	-	-	L	-	-	-	-	-	-	-	-	-	-	V	-	-	-	268	

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Figure 2D

	V													
mouse MSH-R	L	C	L	Y	t	F	F	L	A	M	L	a	208	
human MSH-R	L	C	L	Y	v	F	F	L	A	M	L	i	210	
human ACTH-R	i	t	f	t	s	1	F	P	i	M	v	L	Y	196
rat cannab.	-	-	-	-	-	F	P	L	-	-	-	L	-	289
	V													
mouse MSH-R	F	t	R	A	C	Q	H	v	Q	G	i	A	q	229
human MSH-R	L	a	R	A	C	Q	H	a	Q	G	i	R	Q	231
human ACTH-R	F	-	-	-	-	-	-	-	1	1	A	R	s	210
rat cannab.	-	-	-	-	-	-	-	-	(31)	-	-	R	P	-
	VI													
mouse MSH-R	Q	G	F	s	L	K	G	A	a	T	L	G	i	250
human MSH-R	Q	G	F	g	L	K	G	A	v	T	L	G	i	252
human ACTH-R	p	r	a	n	m	K	G	A	i	T	L	G	v	231
rat cannab.	-	R	-	-	-	A	-	T	L	-	-	L	-	359

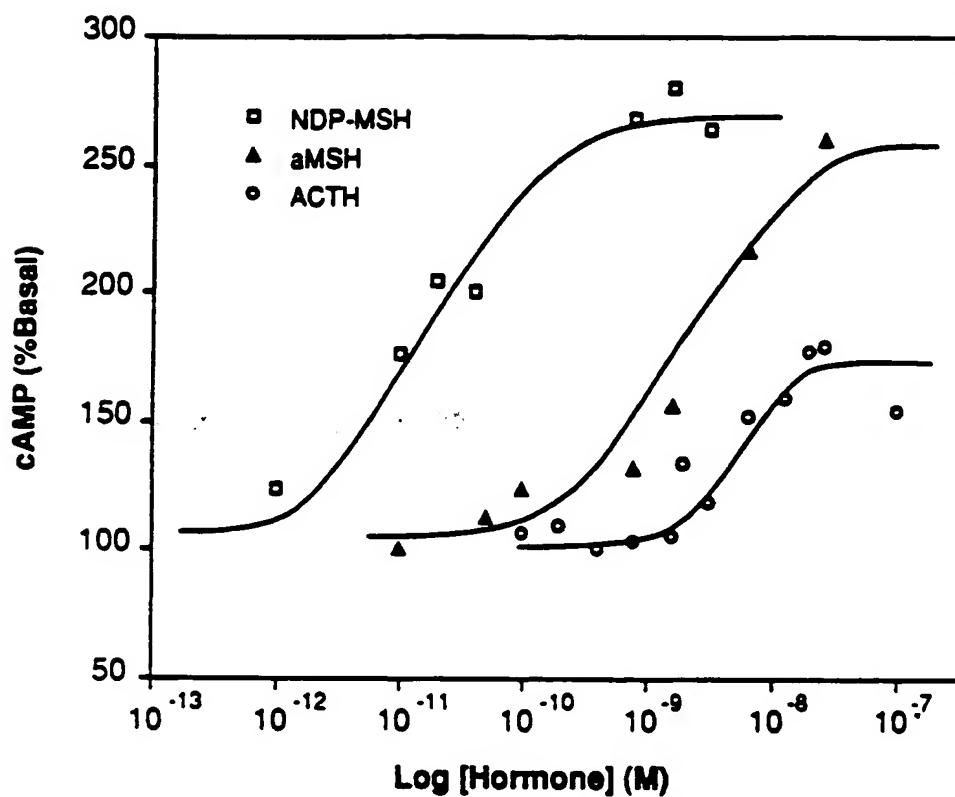
SUBSTITUTE SHEET

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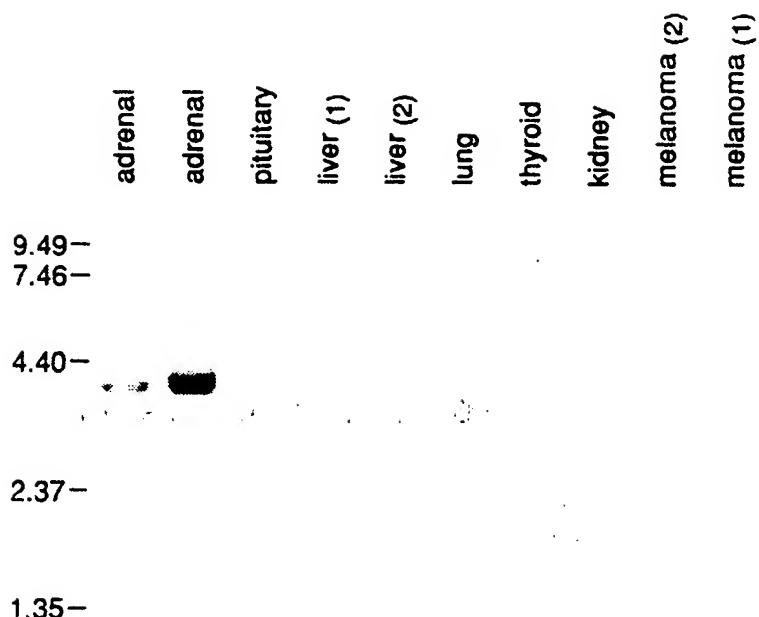
Figure 2E

	VI														VII													
mouse MSH-R	W	G	P	F	L	H	L	L	I	V	L	C	P	q	H	P	T	C	s								271	
human MSH-R	W	G	P	F	L	H	L	L	I	V	L	C	P	e	H	P	T	C	g								273	
human ACTH-R	W	a	P	F	v	L	H	v	L	m	t	f	C	P	s	n	P	y	c	a							252	
rat cannab.	W	P	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								380	
mouse MSH-R	C	I	F	K	N	F	N	L	F	L	I	L	I	v	I	s	s	t	v	D	P	L				292		
human MSH-R	C	I	F	K	N	F	N	L	F	L	a	L	I	i	C	N	A	i	i	D	P	L				294		
human ACTH-R	C	y	m	s	I	F	q	v	n	g	M	L	I	m	C	N	A	v	i	D	P	f				273		
rat cannab.	-	I	-	-	F	-	-	-	M	L	-	-	M	L	-	L	N	S	T	V	-	P	-			401		
mouse MSH-R	I	Y	A	F	R	S	Q	E	L	R	m	T	L	K	E	V	L	I	C	S	--	W				317		
human MSH-R	I	Y	A	F	h	S	Q	E	L	R	r	T	L	K	e	V	L	t	C	S	--	W				316		
human ACTH-R	I	Y	A	F	R	S	P	E	L	R	d	a	f	K	m	i	f	C	S	ry	W				297			
rat cannab.	I	Y	A	-	R	S	-	-	L	R	-	A	F	-	M	-	F	-	S	--	S	--	(56)		483			

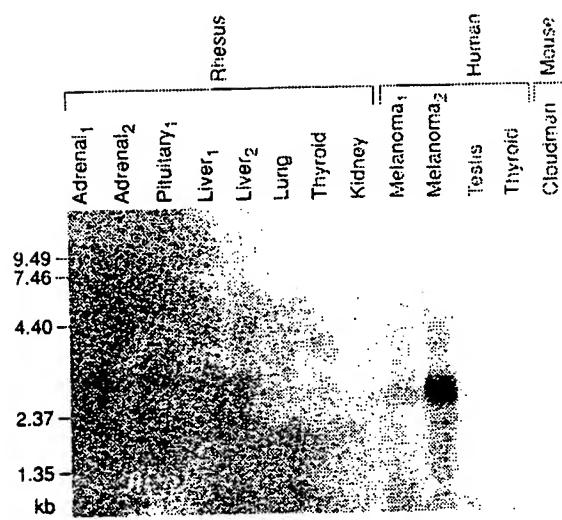
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Figure 3

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Fig. 4

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Figure 4AFigure 4B

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INTERNATIONAL SEARCH REPORT

PCT/US 93/03247

International Application No.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹			
According to International Patent Classification (IPC) or to both National Classification and IPC			
Int.C1. 5	C12N15/12; C12N15/62;	C07K13/00; A61K37/02;	C12P21/08; A61K39/395;
C12N5/10 C12Q1/68			
II. FIELDS SEARCHED			
Minimum Documentation Searched ²			
Classification System		Classification Symbols	
Int.C1. 5	C12N ;	C07K ;	A61K ;
G01N			
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ³			
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁴			
Category ⁵	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²		Relevant to Claim No. ¹³
P,X	SCIENCE vol. 257, 28 August 1992, LANCASTER,PA pages 1248 - 1251 Mountjoy KG;Robbins LS;Mortrud MT;Cone RD; 'The cloning of a family of genes that encode the melanocortin receptors.' see the whole document ---		1-45
P,X	FEBS LETTERS. vol. 309, no. 3, 14 September 1992, AMSTERDAM NL pages 417 - 420 Chhajlani V;Wikberg JE 'Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA.' see the whole document ---		1-45
			-/-
<p>¹⁰ Special categories of cited documents:</p> <ul style="list-style-type: none"> ¹¹ "A" document defining the general state of the art which is not considered to be of particular relevance ¹² "E" earlier document but published on or after the international filing date ¹³ "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) ¹⁴ "O" document referring to an oral disclosure, use, exhibition or other means ¹⁵ "P" document published prior to the international filing date but later than the priority date claimed <p>¹⁶ "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>¹⁷ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>¹⁸ "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>¹⁹ "A" document member of the same patent family</p>			
IV. CERTIFICATION			
2	Date of the Actual Completion of the International Search 15 SEPTEMBER 1993	Date of Mailing of this International Search Report 15.09.93	
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer NAUCHE S.A.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P, X	<p>THE BIOCHEMICAL JOURNAL vol. 286, 1 September 1992, LONDON, GB pages 377 - 382 Ahmed AR; Olivier GW; Adams G; Erskine ME; Kinsman RG; Branch SK; Moss SH; Notarianni LJ; Pouton CW; 'Isolation and partial purification of a melanocyte-stimulating hormone receptor from B16 murine melanoma cells. A novel approach using a cleavable biotinylated photoactivated ligand and streptavidin-coated magnetic beads.' see the whole document ---</p>	1-45
A	<p>JOURNAL OF CELLULAR PHYSIOLOGY vol. 137, no. 1, October 1988, WILEY-LISS, INC. pages 35 - 44 Kameyama K; Montague PM; Hearing VJ; 'Expression of melanocyte stimulating hormone receptors correlates with mammalian pigmentation, and can be modulated by interferons.' see the whole document ---</p>	1-45
A	<p>EUROPEAN JOURNAL OF PHARMACOLOGY vol. 181, no. 1-2, 31 May 1990, pages 71 - 82 Leiba H; Garty NB; Schmidt-Sole J; Piterman O; Azrad A; Salomon Y; 'The melanocortin receptor in the rat lacrimal gland: a model system for the study of MSH (melanocyte stimulating hormone) as a potential neurotransmitter.' see the whole document -----</p>	1-45